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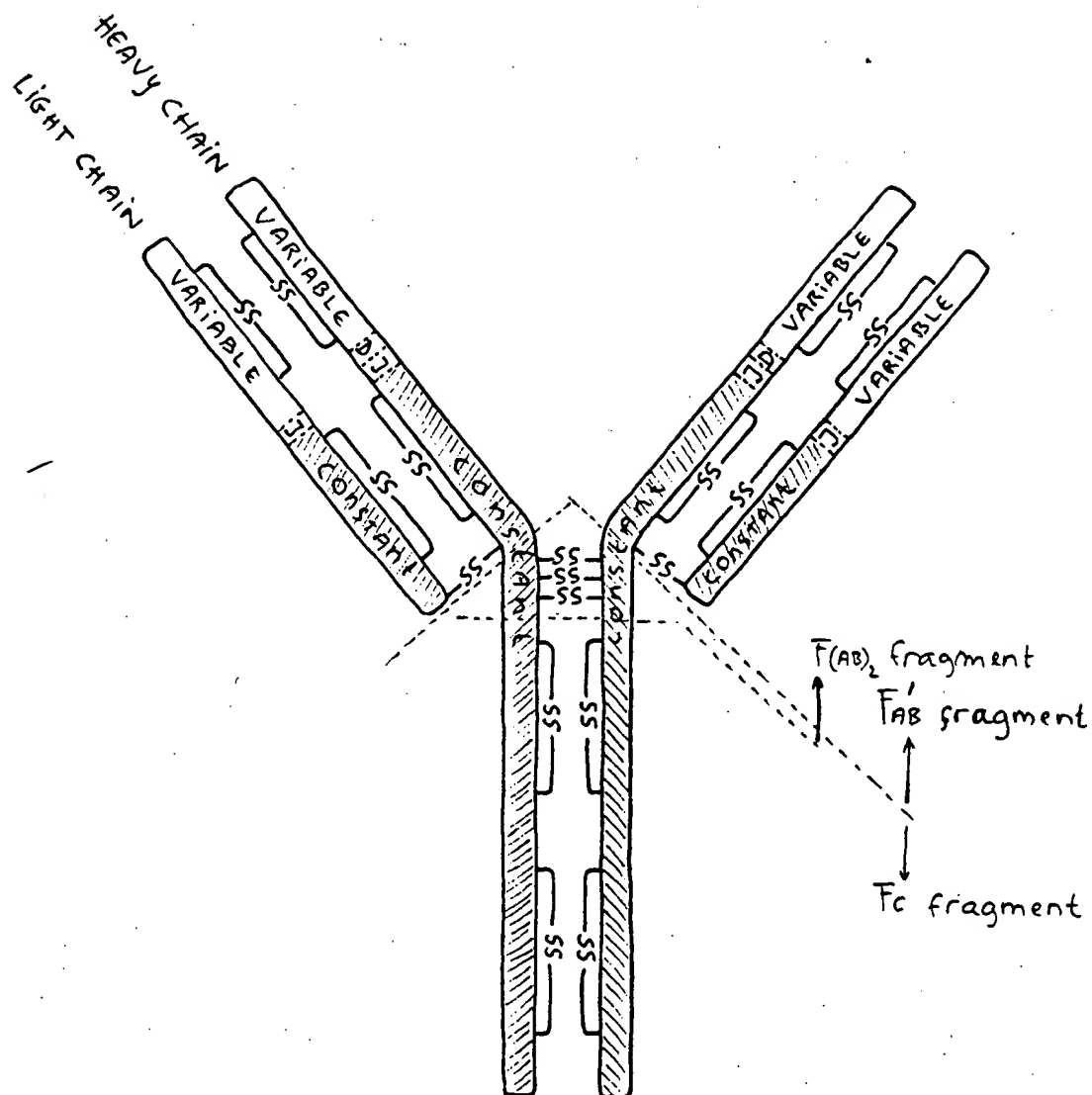


FIGURE 1

Schematic representation of a typical immunoglobulin

1 GTTGTCTGG TTGTCTGGT TTGAAGGAGA CATTGTGATG ACCGAGTCTC ACAAATTCAT GTCCACATCA GTAGGAGACA GGGTCAGCAT CACCTGCAAG
CAACGACACC AACAGACCAC AACTTCTCT GTAACACTAC TGGGTGAGAG TGTTAAGTA CAGGTGTAGT CATCTCTGT CCCAGTCGTA GTGGACGTTT
haeIII haeI

101 GCCAGTCAGG ATGTGGGTGC TGCTATAGCC TGGTATCAAC AGAAACCAGG ACAATCTCCT AAACACTGA TTTACTGGGC ATCCACCCGG CACACTGGAG
CGGTGAGTCC TACACCCACG ACGATATCGG ACCATAGTTG TCTTTGGTCC TGTTAGAGGA TTTGATGACT AAATGACCCG TAGGTGGGCC GTGTGACCTC
fokI sfaNI

201 TCCCTGATCG CTTACAGGC AGTGGATCTG GGACAGATT CACTCTCACC ATTAGCAATG TGCAGTCTGA TGACTTGGCA GATTATTTCT GTCAACAATA
AGGACTAGC GAAGTGTCCG TCACCTAGAC CCTGTCTAAA GTGAGAGTGG TAATCGTTAC ACGTCAGACT ACTGAACCGT CTAATAAAGA CAGTTGTTAT
sau3A xhoI
dnlI sau3A
dnlI hphI hincII

301 TAGCGGGTAT CCTCTCACGT TCGGTGCTGG GACCAAGCTG GAGCTGAAAC GGGGTGATGC TGCACCAACT GTATCCATCT TCCACCATC CAGTGAGCAG
ATCGCCATA GGAGAGTGCA AGCCACGACC CTGGTTCGAC CTCGACTTTC CCCGACTACG ACGTGGTTGA CATAGGTAGA AGGGTGGTAG GTCACTCGTC
mnII sau96
avaII aluI aluI sfaNI fnu4HI hpaI
bbv mboII hincII

401 TTAACATCTG GAGGTGCCTC AGTCGTGTGC TTCTTGAACA ACTTCTACCC CAAAGACATC AATGTCAAGT GGAAGATTGA TGGCAGTGAA CGACAAAATG
AATTGTAGAC CTCCACGGAG TCAGCACAGC AAGAAGTGT TGAAGATGGG GTTCTGTAG TTACAGTTCA CCTCTAATC ACCGTCACCT GCTGTTTTAC
mnII mboII
mnII ddel xmiI

501 GCGTCCTGAA CAGTTGGACT GATCAGGACA GCAAAGACAG CACCTACAGC ATGAGCAGCA CCCTCACGTT GACCAAGGAC GAGTATGAAC GACATAACAG
CGCAGGACTT GTCAACCTGA CTAGTCTGTG CGTTTCTGTC GTGGATGTGC TACTCGTCTG GGGAGTGCAA CTGGTTCCTG CTCATACTTG CTGTATTGTC
sau3A
dnlI fnu4HI
hgaI bclI bbv mnII hincII aluI

601 CTATACCTGT GAGGCCACTC ACAAGACATC AACTTCACCC ATTGTCAAGA GCTTCAACAG GAATGAGTGT TAGAGACAAA GGTCTGAGA CGCCACCACC
GATATGGACA CTCCGGTGAG TGTCTGTAG TTGAAGTGGG TAACAGTTCT CGAAGTTGTC CTTACTCACA ATCTCTGTTT CCAGGACTCT GCGGTGGTGG
mnII haeIII
haeI hphI aluI sau96 hgaI
avaII ddel acyl

701 AGCTCCCCAG CTCCATCCTA TCTTCCCTTC TAAGGTCTTG GAGGCTTCCC CACAAGCGAC CTACCACTGT TCGGCTGCTC CAAACCTCCT CCCCACCTCC
TCGAGGGGTC GAGGTAGGAT AGAAGGGAAG ATTCCAGAAC CTCGGAAGGG GTGTTCTGCTG GATGGTGACA ACGCCACGAG GTTTGGAGGA GGGGTGGAGG
fokI mnII
mnII ddel mnII hgiA mnII mnII

801 TTCTCTCTCT CTTCCCTTTC CTTGGCTTTT ATCATGCTAA TATTTGCAGA AAATATTCAA TAAAGTGAGT CTTTGCATT GA
AAGAGGAGGA GGAGGGAAG GAACCGAAAA TAGTACGATT ATAAAGTCT TTTAAGTT ATTCACTCA GAAACGTGAA CT
mnII xmiI hincII

nucleotides: 882

FIGURE 2

Nucleotide sequence of pK17G4 cDNA insert, including
~~light (non-coding) region.~~

FIGURE 3
Light (Kappa) chain amino acid sequence and codons

1
 hinfI sau96 ddel
 GAGTCAGCAC TGAACAGCGA CCGCTCACC AACTTCGG GCTCAGCTTG ATTTACCTTG TCCTTGTTT AAGTTGTC CAGTGGAAG TGAAGTCTG
 CTGAGTCGTG ACTTGTCCT GGGGAGTGT ACTTGAAGCC CGAGTCGAAC TAAATGGAAC AGGAACAAAA TTTTCAACAG GTCACACTTG AAGTCTG

101
 hinfI hinfI ecorII auaI fnu4HI hinfI
 GGAGTCCTGG GGAGTCTTAA TGGAGCCTGG AGGGTCCCTG AAACCTCTCT GTGACGCTC TGGATTCACT TTCAGTAGAT ATGCCATGTC TTGGGTTCGC
 CCTCAGACCC CCTCAGAATT ACCTCGGACC TCCAGGGAC TTTGAGAGGA CACGTGCGAG ACCTAAGTGA AAGTCATCTA TACGGTACAG AACCAAGCG

201
 hpaII mnlI hinfI mbolI hpaII hinfI
 CAGACTCCGG AGAAGAGGCT GGAGTGGGTC GCAACCATTG GTAGTGGTGG TAGTTCACAC CTTCATCCA GACAGTGTGA AGGGCGATTG ACCATCTCCA
 GTCTGAGGCC TCTTCTCGA CCTCACCAG CGTTGGTAAT CATCACCACC ATCAAGTGTG GAAGGTAGGT CTGTCACACT TCCCGCTAAG TGGTAGAGGT
 foki

301
 rsal mnlI mnlI ddel haeIII
 GAGACAATGC CAAGAACCCT CTGACCTGC AAATGAGCAG TCTGAGGCTT GAGGACACGG CCAATGTATTA CTGTGCAAGA CCCCCTCTTA TTTCTGTAGT
 CTCTGTTACG GTTCTGTGG GACATGGAGC TTTACTGCTC AGACTCCAGA CTCTGTGCC GGTACATAAT GACACGTTCT GGGGGAGAAT AAGCAATCA

401
 mnlI ddel hpaII mnlI ddel haeIII
 AGCGGACTAT GCTATGGACT ACTGGGGTCA AGGAACCTCA GTCACCGTCT CCTCAGCCAA AACGACACCC CCATCTGTCT ATCCACTGGC CCGTGGATCT
 TCGCCTGATA CGATACCTGA TGACCCAGT TCTTGGAGT CAGTGGCAGA GGAGTCGGT TTGCTGTGGG GGTAGACAGA TAGGTGACCG GGGACCTAGA

501
 fnu4HI ncoI sfaNI foki hpaII ecorII scrFI haeIII
 GGTGCCCCAA CTAATCCAT GGTGACCCCTG GGATGCTGG TCAAGGGCTA TTTCCCTGAG CCAGTGACAG TGACCTGGAA CTCTGGATCC CTGTCCAGCG
 CGACGGGTTT GATTGAGTA CCACTGGGAC CTAACGGACC AGTTCGCCAG AAAGGGACTC GGTCACTGTC ACTGGACCTT GAGACCTAGG GACAGGTGCG

601
 hgaI pvuII aluI pstI mnlI ddel aluI mnlI haeIII hpaII
 GTGTGCACAC CTTCCAGCT GTCTGCGT CTGACCTCTA CACTCTGAGC AGCTCAGTGA CTGTCCCTC CAGCCCTCGG CCCAGCGAGA CCGTCACCTG
 CACACGTGTG GAAGGGTCGA CAGGACGTCA GACTGGAGAT GTGAGACTCG TCGAGTCACT GACAGGGGAG GTCGGGAGCC GGGTGGCTCT GGCAGTGGAC

701
 scrFI haeIII nciI fnu4HI hpaII haeIII
 CAACGTTGCC CACCCGGGCA GCAGCACCAA GGTGGACAAG AAAATTGTGC CAGGGGATTG TGGTTGTAAG CTTTGCATAT GTACAGTCCC AGAAGTATCA
 GTTGCAACGG GTGGGCCGGT CGTCGTGGTT CCACCTGTT TTTTAAACAG GGTCCCTAAC ACCAACATTC GGAACGTATA CATGTCAGGG TCTTCATAGT

801
 mbolI mbolI foki hgaI hpaII hinfI mstII ddel accI foki auaI
 TCTGTCTTCA TCTTCCCCC AAAGCCCCAG GATGTGCTCA CCATTACTCT GACTCTCAAG GTCACGTGTG TTGTGGTAGA CATCAGCAAG GATGATCCCG
 AGACAGAAGT AGAAGGGGGG TTTGCGGTTT CTACACGAGT GGTAAATGAGA CTGAGGATTC CAGTGCACAC AACACCATCT GTAGTCGTTT CTACTAGGGC

901
 sau96 pvuII mnlI ddel hgaI aluI hgaI auaI mnlI ddel
 AGTCCAGTT CAGCTGGTTT GTAGATGATG TGGAGGTGCA CACAGCTCAG ACGCCAACC GGGAGGAGCA GTTCAACAGC ACTTCCCGCT CAGTCAGTGA
 TCCAGGTCAA GTCGACCAAA CATCTACTAC ACCTCCAGCT GTGTCGAGTC TCGTTGGGG CCGTCTCTGT CAAGTTGTG TGAAGGGCGA GTCAGTCACT

1001
 scrFI ecorII hincII aluI hincII taqI
 ACTTCCATC ATGCACCAGG ACTGGCTCAA TGGCAAGGAG TTCAATGCA GGTCAACAG TGCAGCTTTC CCTGCCCCCA TCGAGAAAAA CATCTCCAAA
 TGAAGGGTAG TACGTGGTCC TGACCGAGTT ACCGTTCTCT AAGTTTACGT CCCAGTTGTC ACCTGCAAGG GACGGGGGT AGCTCTTTTG GTAGAGGTTT

1101
 haeIII haeI haeI
 ACCAAAGGCA GACCGAAGGC TCCACAGGTG TACACCATTG CACTCCCAA GGAGCAGATG GCCAAGGATA AAGTCAGTCT GACCTGCATG ATAACAGACT
 TGGTTTCCGT CTGGCTCCG AGGTGTCCAC ATGTGGTAAG GTGGAGGGTT CCGTCTCTAC CGGTTCTAT TTCACTCAGA CTGGACGTAC TATTGTCTGA

1201
 mbolI mbolI fnu4HI hpaII haeIII
 TCTTCCCTGA AGACATTACT GTGGAGTGGC AGTGAATGG GCAGCCAGCG GAGAACTACA AGAACAATCA GCCCATCATG AACACGAATG GCTCTTACTT
 AGAAGGGACT TCTGTAATGA CACCTCACCG TCACCTTACC CAGCGGTGCG CTCTGTGATG TCTGTGAGT CCGGTAGTAC TGTGCTTAC CGAAGATGAA

1301
 accI aluI mbolI mnlI hpaII haeIII ddel
 CGTCTACAGC AAGCTCAATG TGCAGAAGAG CAATGGGAG GCAGGAAATA CTTTCACTG CTCTGTGTTA CATGAGGGCC TGCACAACA CCATCTGAG
 GCAGATGTG TTAGAGTTAC AGTCTTCTC GTTGACCTC CGTCTTTAT GAAAGTGGAC GAGACACAAT GTACTCCCGC AGGTGTTGGT GGTATGACTC

1401
 mnlI scrFI sau3A hpaII haeIII
 AAGAGCTCT CCCACTCTCC TGGTAAATGA TCCAGTGTG CTTGGAGCCC TCTGGTCTTA CAGGACTCTG ACACCTACCT CCACCCCTCC CTGTATAAAT
 TTTCCGAGA GGGTGAAGG ACCATTACT AGGGTCACAG GAACCTCGGG AGACAGGAT GTCTGAGAC TGTGGATGGA GGTGGGGAGG GACATATTTA

1501
 AAAGCACCCA GCACTGCTT GGGAAAAA
 TTTCTGGGT CGTGACGGAA CCGTTTTT

FIGURE 4

p gamma 298 cDNA insert and p γ 11 cDNA insert ligation containing coding sequence for heavy (gamma) anti CEA chain

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-10

met asn phe gly leu ser leu ile tyr leu val leu val leu lys val val gln cys glu
 GAGUCAGCACUGAACACGGACCCUCACG AUG AAC UUC GGG CUC AGC UUG AUU UAC CUU GUC CUG GUU UUA AAA GUU GUC CAG UGU GAA

10 20 30
 val met leu val glu ser gly gly val leu met glu pro gly gly ser leu lys leu ser cys ala ala ser gly phe thr phe ser arg
 GUG AUG CUG GUG GAG UCU GGG GGA GUC UUA AUG GAG CCU GGA GGG UCC CUG AAA CUC UCC UGU GCA GCC UCU GGA UUC ACU UUC AGU AGA

40 50 60
 tyr ala met ser trp val arg gln thr pro glu lys arg leu glu trp val ala thr ile ser ser gly gly ser ser his leu pro ser
 UAU GCC AUG UCU UGG GUU CGC CAG ACU CCG GAG AAG AGG CUG GAG UGG GUC GCA ACC AUU AGU AGU GGU GGU AGU UCA CAC CUU CCA UCC

70 80 90
 arg gln cys glu gly arg phe thr ile ser arg asp asn ala lys asn thr leu tyr leu gln met ser ser leu arg ser glu asp thr
 AGA CAG UGU GAA GGG CGA UUC ACC AUC UCC AGA GAC AAU GCC AAG AAC ACC CUG UAC CUG CAA AUG AGC AGU CUG AGG UCU GAG GAC AGC

100 110 120
 ala met tyr tyr cys ala arg pro leu ile ser leu val ala asp tyr ala met asp tyr trp gly gln gly thr ser val thr val
 GCC AUG UAU UAC UGU GCA AGA CCC CCU CUU AUU UCG UUA GUA GCG GAC UAU GCU AUG GAC UAC UGG GGU CAA GGA ACC UCA GUC ACC GUC

130 140 150
 ser ser ala lys thr thr pro pro ser val tyr pro leu ala pro gly ser ala ala gln thr asn ser met val thr leu gly cys leu
 UCC UCA GCC AAA ACG ACA CCC CCA UCU GUC UAU CCA CUG GCC CCU GGA UCU GCU GCC CAA ACU AAC UCC AUG GUG ACC CUG GGA UGC CUG

160 170 180
 val lys gly tyr phe pro glu pro val thr val thr trp asn ser gly ser leu ser ser gly val his thr phe pro ala val leu gln
 GUC AAG GGC UAU UUC CCU GAG CCA GUG ACA GUG ACC UGG AAC UCU GGA UCC CUG UCC AGC GGU GUG CAC ACC UUC CCA GCU GUC CUG CAG

190 200 210
 ser asp leu tyr thr leu ser ser val thr val pro ser ser pro arg pro ser glu thr val thr cys asn val ala his pro ala
 UCU GAC CUC UAC ACU CUG AGC AGC UCA GUG ACU GUC CCC UCC AGC CCU CGG CCC AGC GAG ACC GUC ACC UGC AAC GUU GCC CAC CCG GCC

220 230 240
 ser ser thr lys val asp lys lys ile val pro arg asp cys gly cys lys pro cys ile cys thr val pro glu val ser ser val phe
 AGC AGC ACC AAG GUG GAC AAG AAA AUU GUG CCC AGG GAU UGU GGU UGU AAG CCU UGC AUA UGU ACA GUC CCA GAA GUA UCA UCU GUC UUC

250 260 270
 ile phe pro pro lys pro lys asp val leu thr ile thr leu thr pro lys val thr cys val val val asp ile ser lys asp asp pro
 AUC UUC CCC CCA AAG CCC AAG GAU GUG CUC ACC AUU ACU CUG ACU CCU AAG GUC ACG UGU GUU GUG GUA GAC AUC AGC AAG GAU GAU CCC

280 290 300
 glu val gln phe ser trp phe val asp asp val glu val his thr ala gln thr gln pro arg glu glu gln phe asn ser thr phe arg
 GAG GUC CAG UUC AGC UGG UUU GUA GAU GAU GUG GAG GUG CAC ACA GCU CAG ACG CAA CCC CGG GAG GAG CAG UUC AAC AGC ACU UUC CGC

310 320 330
 ser val ser glu leu pro ile met his gln asp trp leu asn gly lys glu phe lys cys arg val asn ser ala ala phe pro ala pro
 UCA GUC AGU GAA CUU CCC AUC AUG CAC CAG GAG UGG CUC AAU GGC AAG GAG UUC AAA UGC AGG GUC AAC AGU GCA GCU UUC CCU GCC CCC

340 350 360
 ile glu lys thr ile ser lys thr lys gly arg pro lys ala pro gln val tyr thr ile pro pro pro lys glu gln met ala lys asp
 AUC GAG AAA ACC AUC UCC AAA ACC AAA GGC AGA CCG AAG GCU CCA CAG GUG UAC ACC AUU CCA CCU CCC AAG GAG CAG AUG GCC AAG GAU

370 380 390
 lys val ser leu thr cys met ile thr asp phe phe pro glu asp ile thr val glu trp gln trp asn gly gln pro ala glu asn tyr
 AAA GUC AGU CUG ACC UGC AUG AUA ACA GAC UUC UUC CCU GAA GAC AUU ACU GUG GAG UGG CAG UGG AAU GGG CAG CCA GCG GAG AAC UAC

400 410 420
 lys asn thr gln pro ile met asn thr asn gly ser tyr phe val tyr ser lys leu asn val gln lys ser asn trp glu ala gly asn
 AAG AAC ACU CAG CCC AUC AUG AAC ACG AAU GGC UCU UAC UUC GUC UAC AGC AAG CUC AAU GUG CAG AAG AGC AAC UGG GAG GCA GGA AAU

430 440 447
 thr phe thr cys ser val leu his glu gly leu his asn his his thr glu lys ser leu ser his ser pro gly lys OP
 ACU UUC ACC UGC UCU GUG UUA CAU GAG GGC CUG CAC AAC CAC CAU ACU GAG AAG AGC CUC UCC CAC UCU CCU GGU AAA UGA UCCGAGUGUCCU
 UGGAGCCUCUGGUCCUACAGGACUCUGACACCUCCACCCUCCUUAUAAAAAAGACCCAGCACUGCCUUGGAAAAA

FIGURE 5

Amino acid and coding sequence for heavy (gamma-1)
 anti CEA chain

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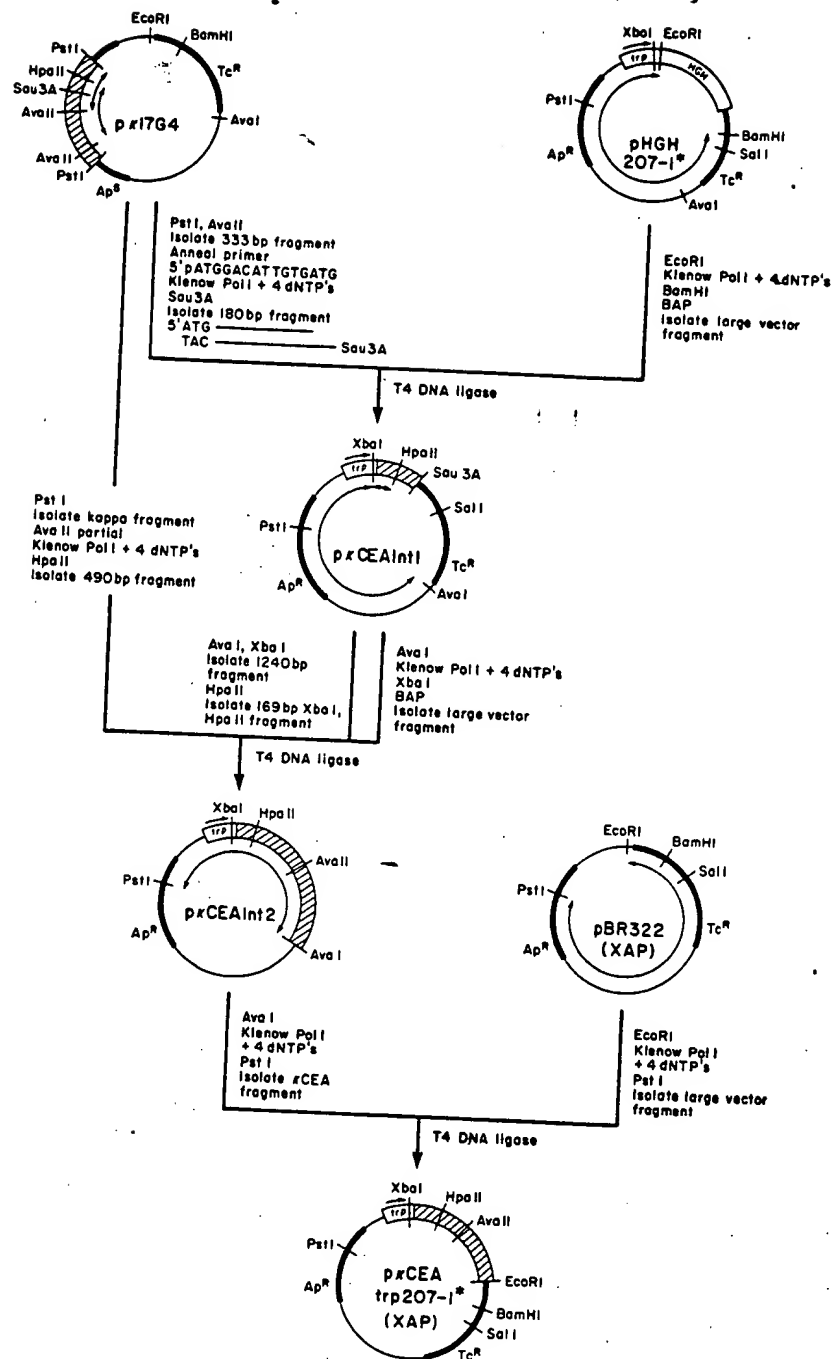


FIGURE 6

Construction of plasmid for direct expression of light anti CEA (kappa) chain gene

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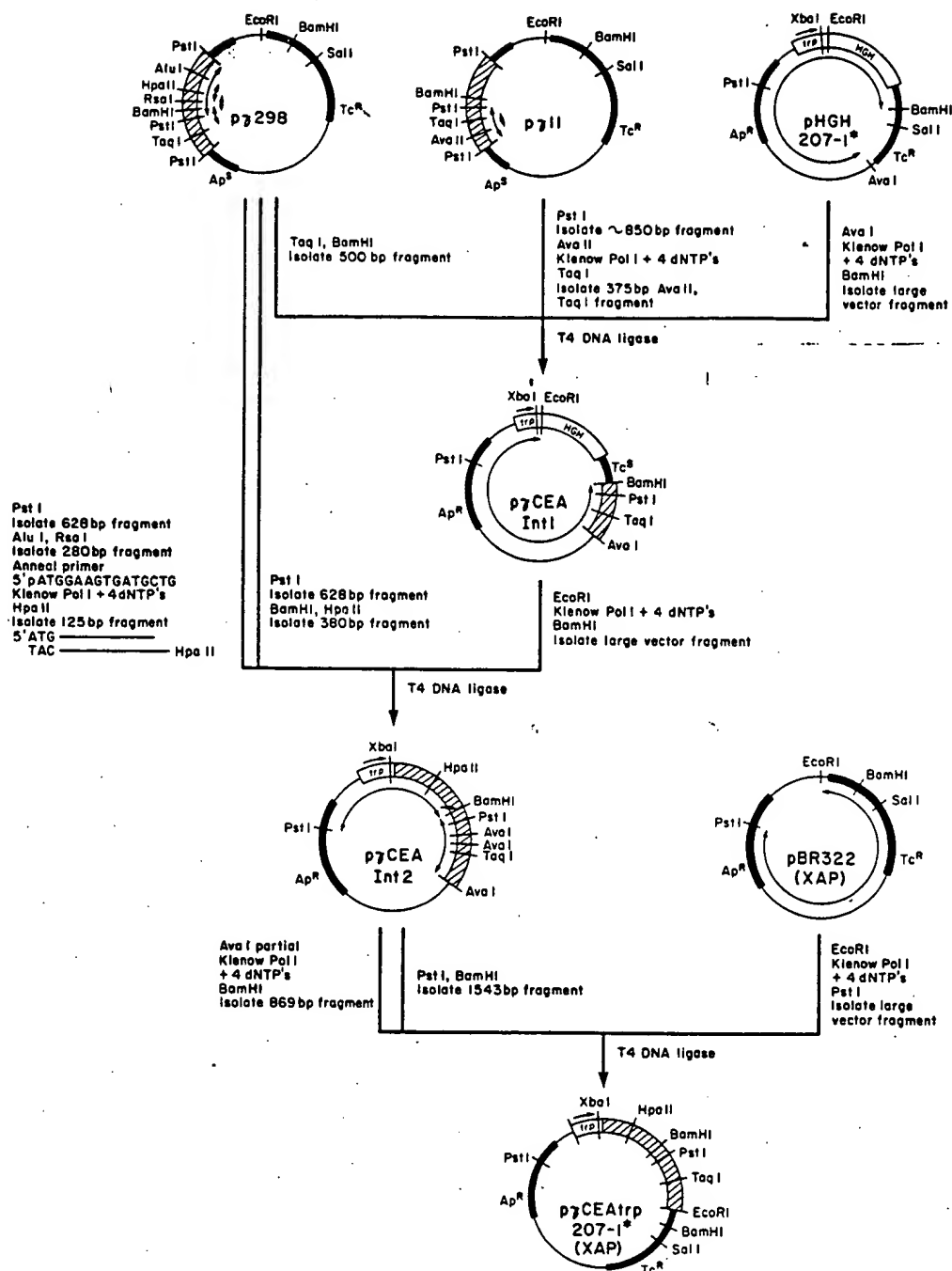


FIGURE 7

Construction of plasmid for direct expression of anti CEA light (gamma 1) chain

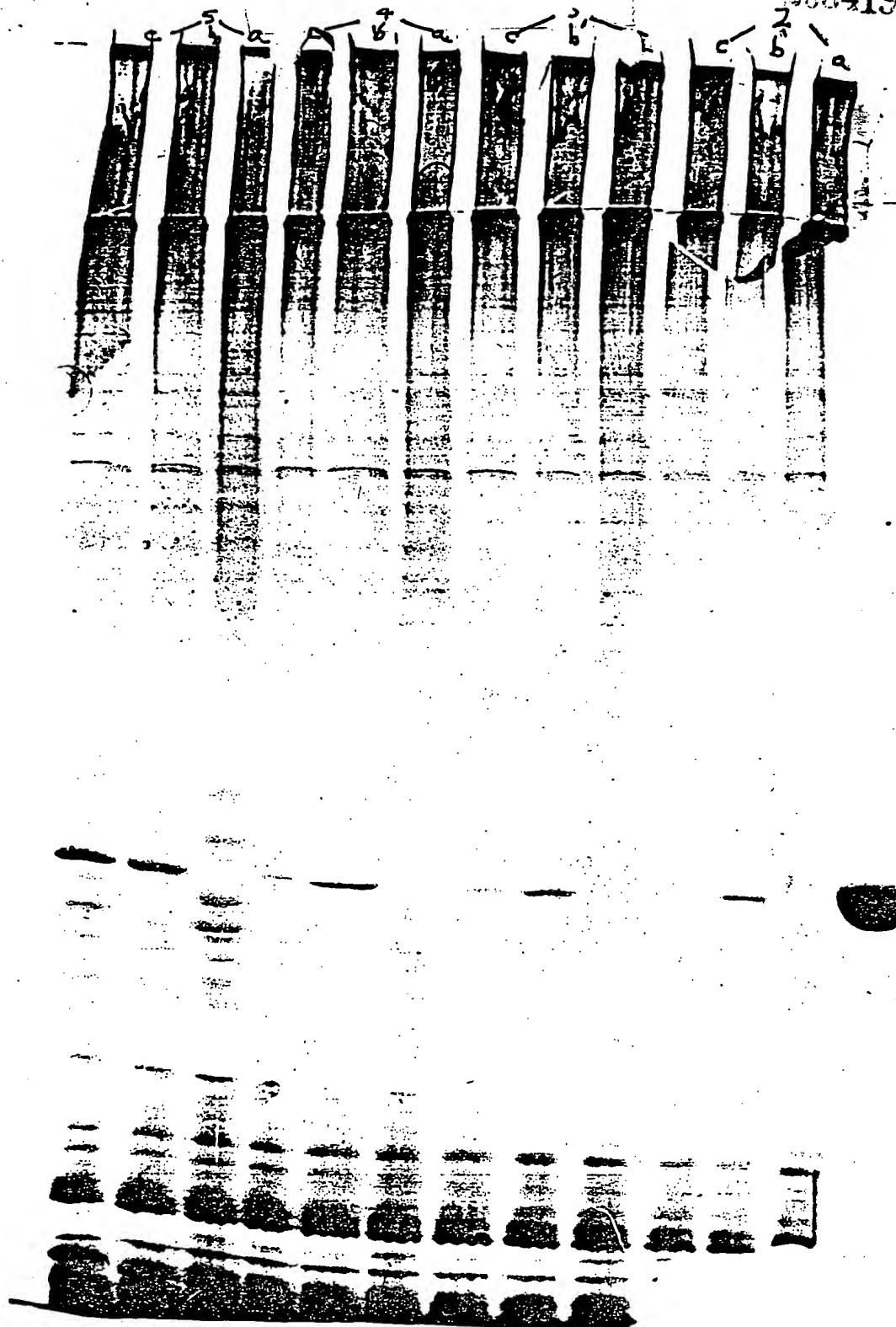


FIGURE 8A
Silver stained SDS-PAGE of extracts from E. coli transformed with pYCEAt_{trp}207-

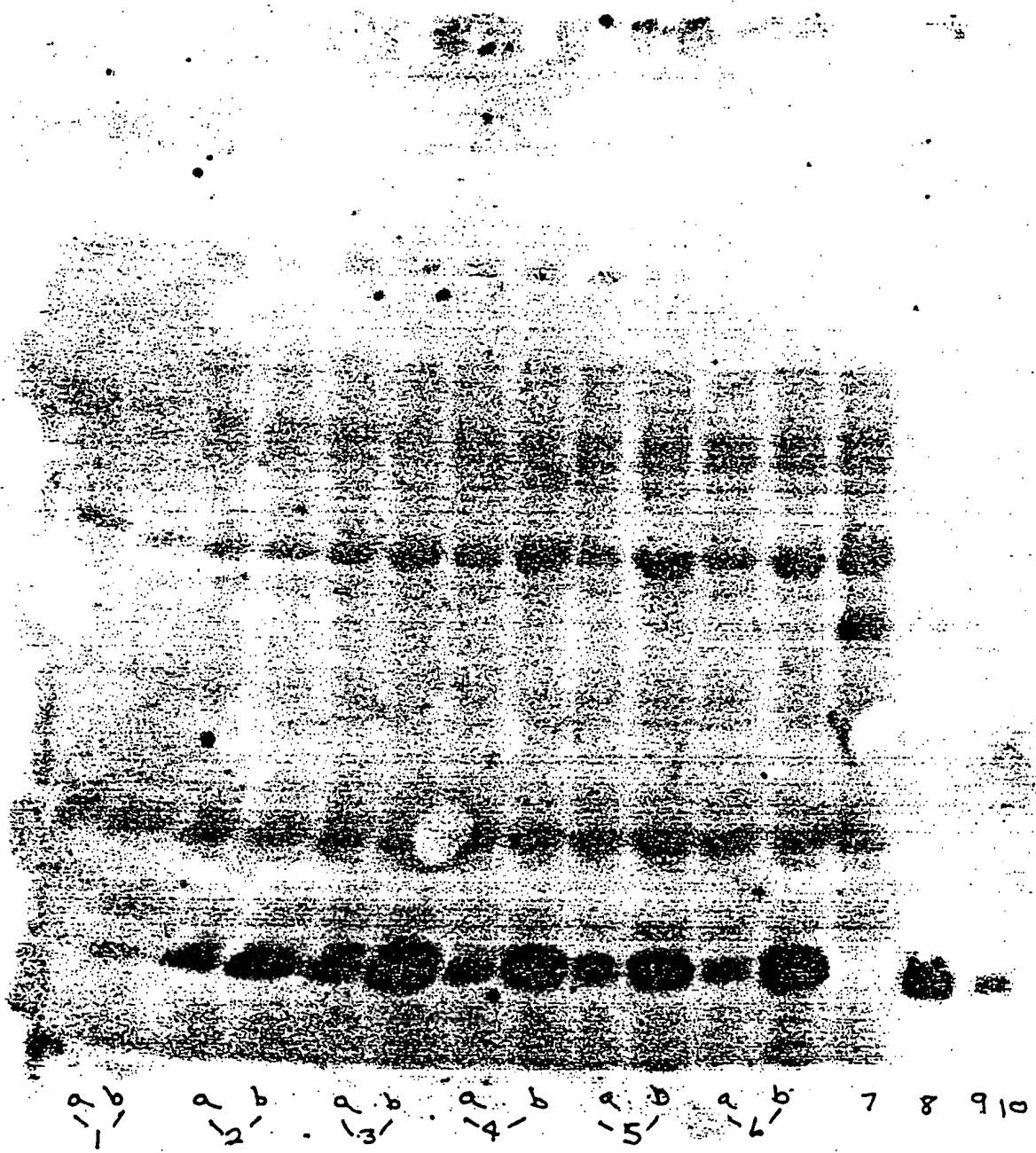


FIGURE 8B

Western blot of SDS PAGE performed on extracts of E.coli transformed with PKCEAtrp207-1*

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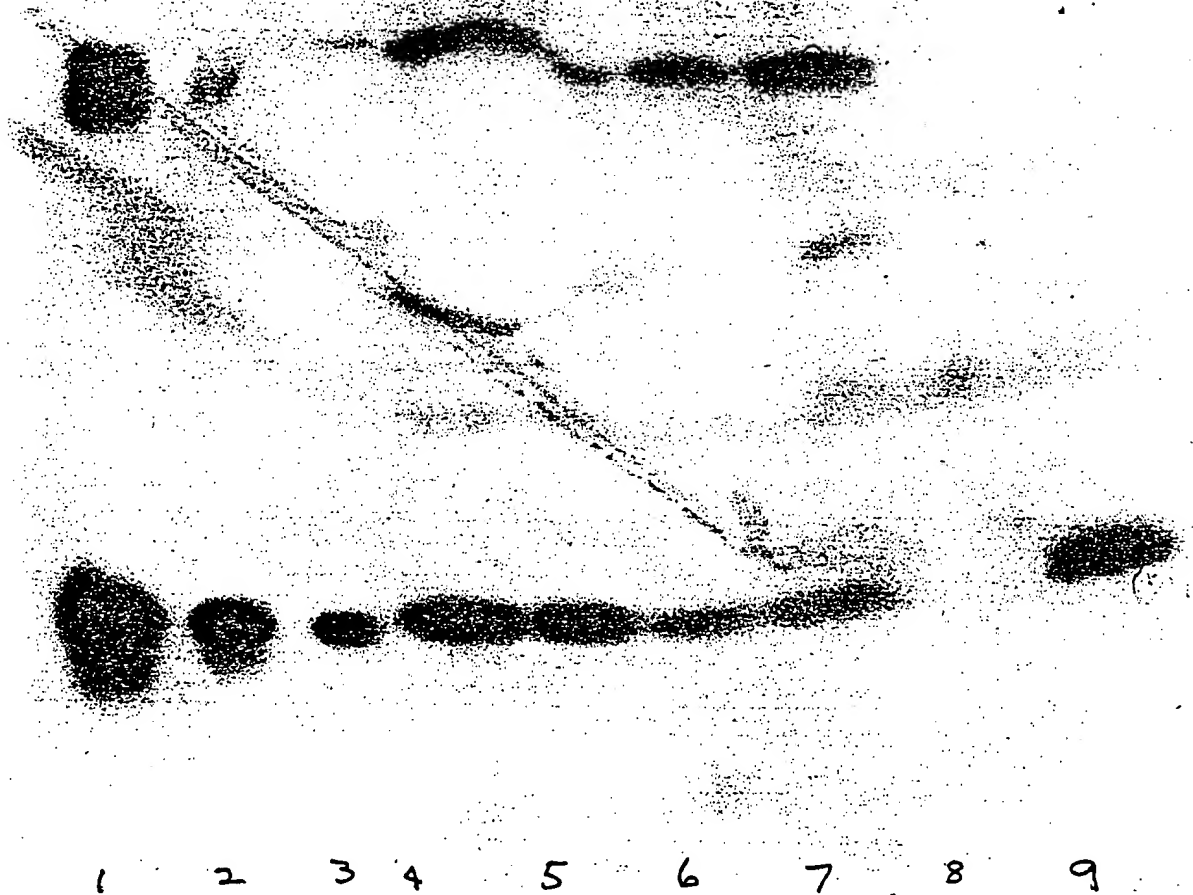


FIGURE 8C

Western blot of SDS PAGE E. coli double transformed with
pKCEAtrp207-1*4 and pyCEAInt2.

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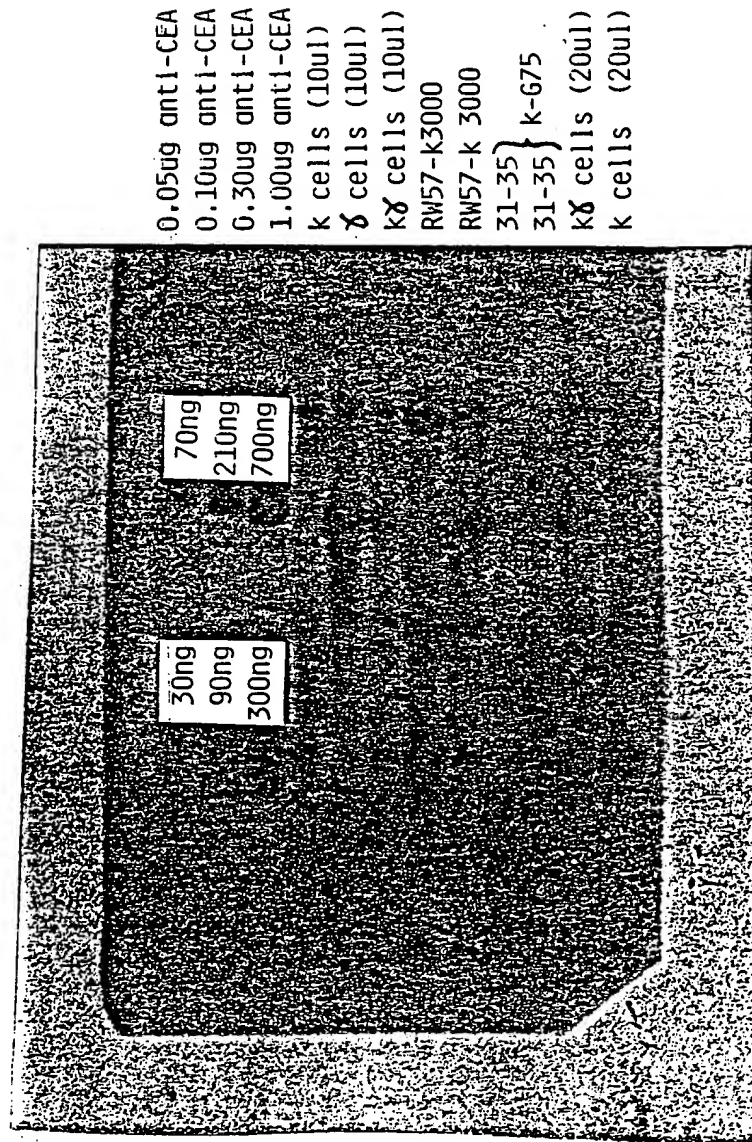


FIGURE 9

Western blot of SDS PAGE performed on extracts of *E. coli* transformed with plasmids containing DNA sequences and coding heavy light and both heavy and light anti CEA chains.

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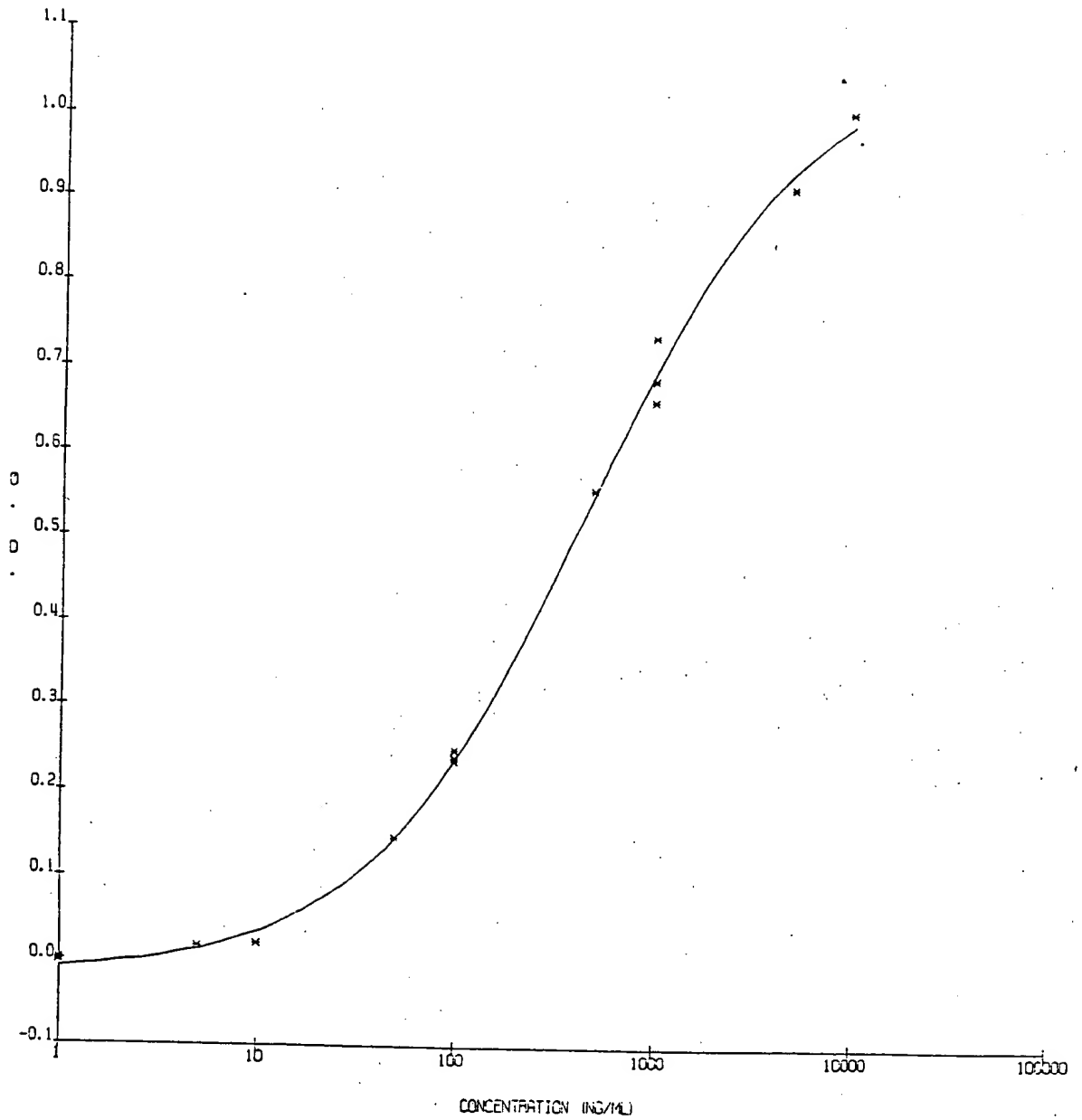


FIGURE 10

Standard curve showing dependence of OD on concentration of antibody by ELISA assay

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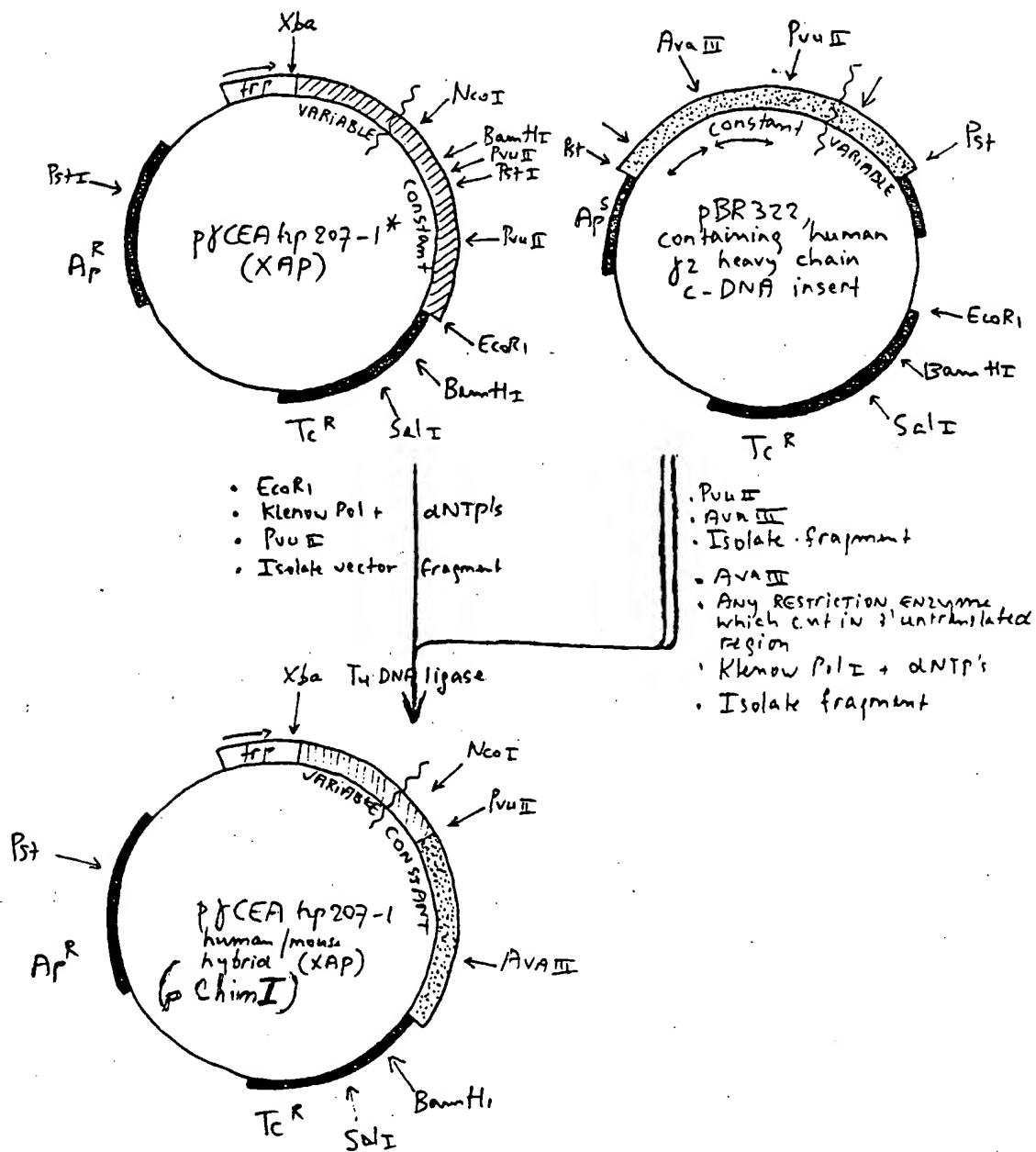


FIGURE 11

Construction of a plasmid for direct expression of chimeric human/murine heavy chain gene

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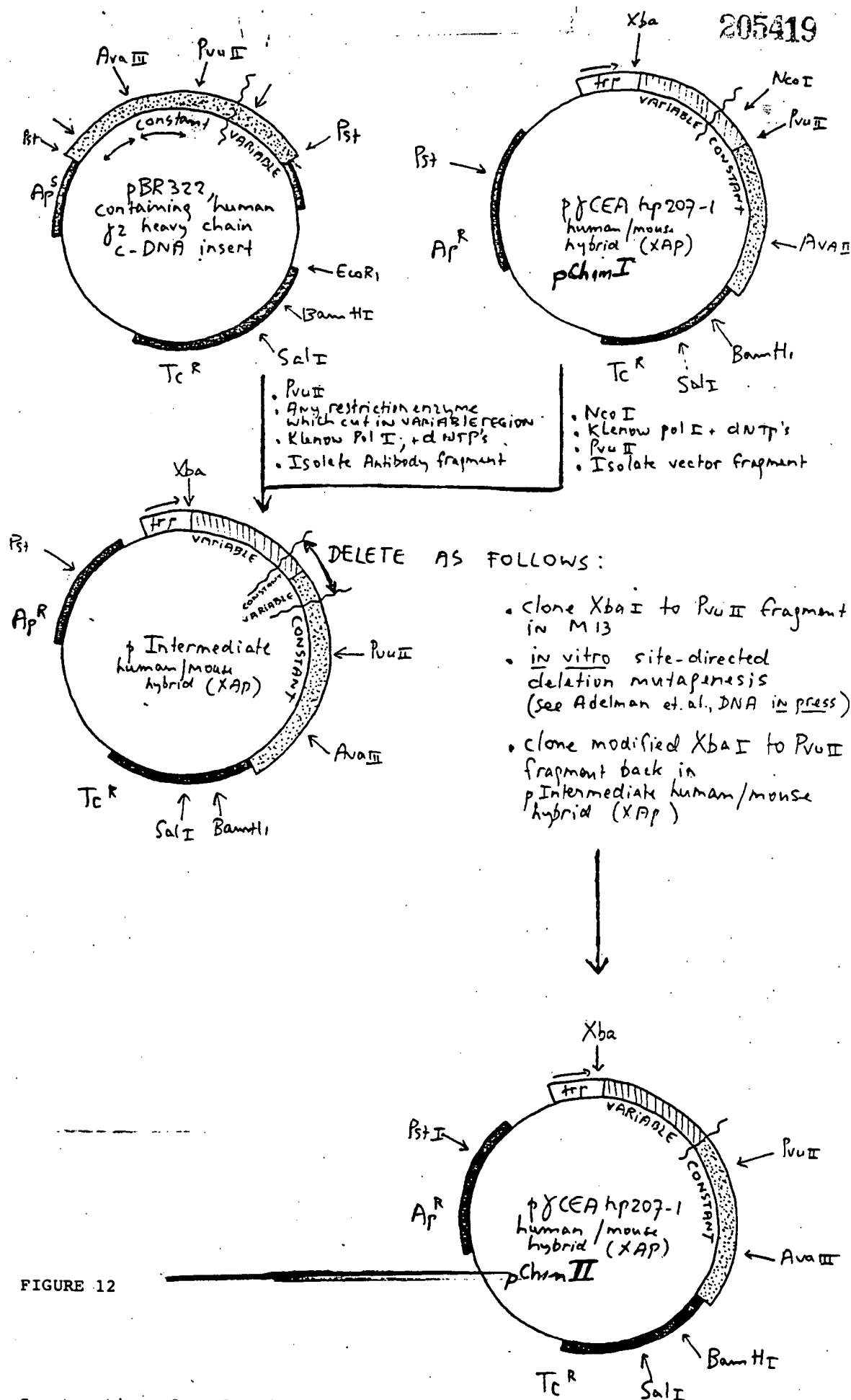


FIGURE 12

Construction of a plasmid for direct expression of chimeric constant/murine variable heavy chain gene

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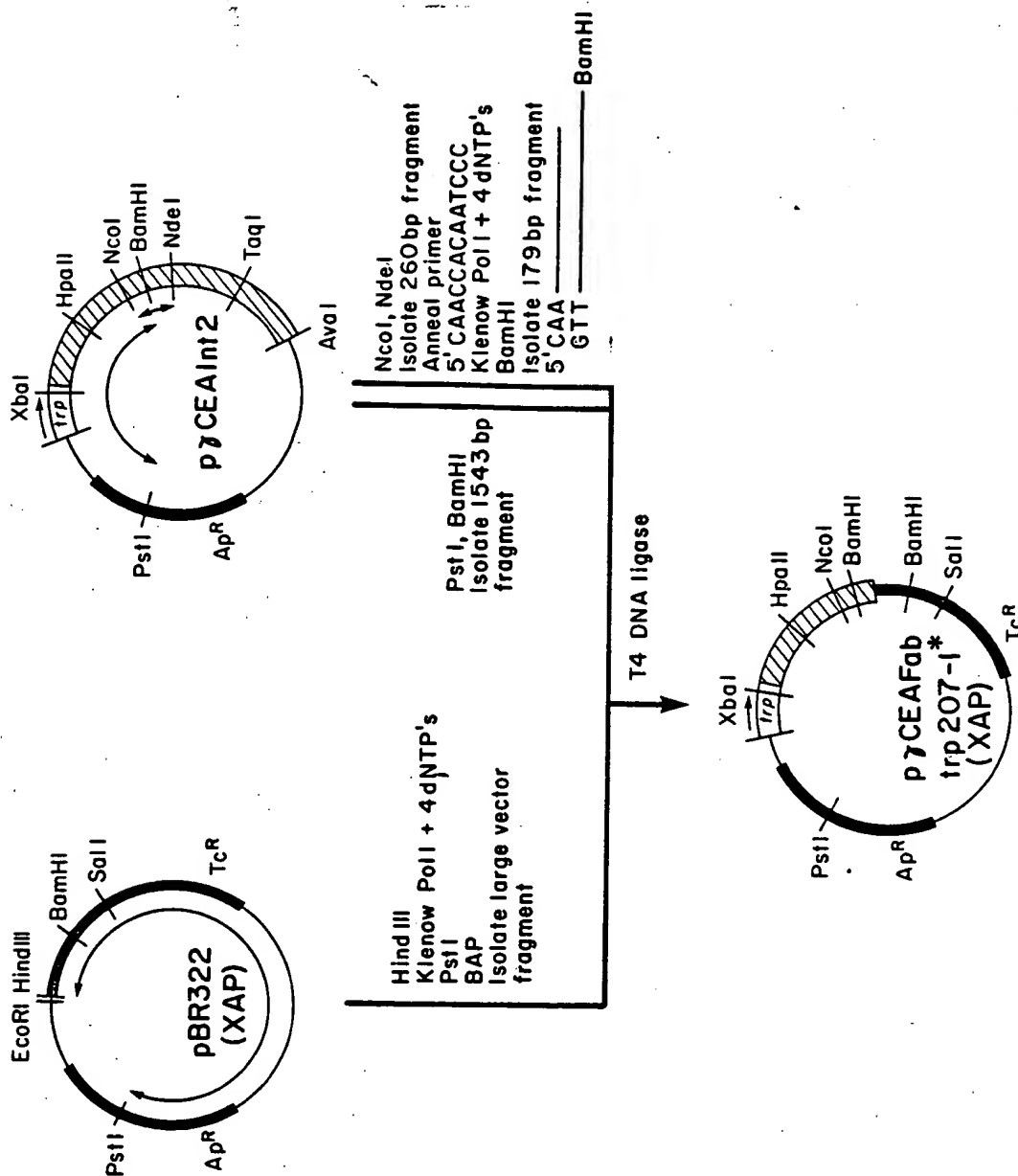


FIGURE 13

Construction of plasmid for direct expression of Fab region from heavy anti CEA chain gene

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